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Investigating the Response of Magnetotactic Bacteria to Varying Field Strength and Developing Autonomous Analysis of Spatial Dispersal

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**Investigating the Response of Magnetotactic Bacteria to Varying Field Strength and
Developing Autonomous Analysis of Spatial Dispersal**

A Thesis

Submitted to the Department of Biology and Biomedical Engineering

of the

Rose-Hulman Institute of Technology

by

Madeleine Dallas Pasco

In partial fulfillment of the requirements for the degree of

Bachelor of Science in Biology

February 2018

Approved: _____
Jennifer O'Connor, Ph.D., Advisor

ABSTRACT

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“Investigation of the Response of Magnetotactic Bacteria to Varying Field Strength and Development of Autonomous Analysis of Spatial Dispersal”

Thesis Advisor: Jennifer O’Connor, Ph.D.

Magnetotactic bacteria (MTB) are single-celled organisms which contain organelles called “magnetosomes,” membrane-bound ferrous nanocrystals. These organelles allow for magnetotaxis, which is movement guided by magnetic fields. MTB are generally found in the top layers of sediment of aqueous environments, and magnetotaxis is thought to help guide these microbes to ideal oxygen concentrations in the water after they may have been displaced by turbulent waters. The magnetic component of this magneto-aerotaxis is thought to be guided by the ambient magnetic field of the Earth (0.25-0.65 gauss). In order to investigate how the strength of the magnetic field affects magnetotaxis, I took images of *Magnetospirillum magneticum*, strain AMB-1 while varying the strength of an induced magnetic field controlled by the distance between permanent bar magnets (2 cm, 78 ± 4 gauss and 10 cm, 6.0 ± 1 gauss). The numbers of bacteria accumulated near the magnets after five minutes of exposure to a field were compared to those of *E. coli*, which has no magnetotactic character. In order to attempt to eliminate the error associated with manual counting of microbes, I created an autonomous program using MATLAB which converts RGB images of bacteria into binary images and counts

the black pixels as a proxy for counting the individual bacteria. The results of both manually and autonomously counting bacteria were compared. Neither counting method indicated a statistically significant effect of the strength of the magnetic field on the net movement of the bacteria. Additionally, in order for the autonomous counting to be useful in future research, more work must be done on how to reliably acquire high-contrast, in-focus images of the MTB.

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2 INTRODUCTION

The term “magnetotactic bacteria” (MTB) encompasses members of a variety of taxa who are able to navigate via the magnetic field of the Earth. These microbes were first discovered by Richard Blakemore in 1975 (Blakemore *et al.*, 1975). They are generally Gram-negative members of the domain Bacteria, motile, non-fermenting, and microaerophilic, and have range of morphologies including coccoid (circular), bacilloid (rod-shaped), vibrioid (curved rod), spirilloid (helical), and multicellular, and are ubiquitous around the world in the top layer of sediments residing at the bottom of bodies of water such as rivers and lakes (Blakemore, 1982; Bazylinski *et al.*, 2004; Perantoni *et al.*, 2009).

Magnetotaxis is considered to be a magnetically-guided form of aerotaxis which guides MTB toward ideal aerobic conditions after they may have been jostled out of their ideal microaerophilic environment by turbulent currents (Blakemore *et al.*, 1980; Smith *et al.*, 2006; Lefèvre *et al.*, 2014; Mao *et al.*, 2014). The field at Earth’s magnetic south pole (geographic North Pole) goes down into the surface of the Earth, whereas the magnetic field at the magnetic north pole (geographic South Pole) points up; this up-down orientation leads to a tilting up or down depending on the geographic Hemisphere and magnetic pole in question, and MTB are thought to move upwards or downwards accordingly in search of ideal oxygen concentrations (Mao *et al.*, 2014; Smith *et al.*, 2006).

The internal structures responsible for this behavior are organelles called “magnetosomes,” which are membrane-bound, ferrous nanocrystals of high chemical purity, usually Fe_3O_4 or Fe_3S_4 (Bazylinski *et al.*, 2004; Faivre & Schüler, 2008). Magnetosomes are arranged in a chain across the long axis of the bacteria (Bazylinski *et al.*, 2004) and are anchored in place by a cytoskeleton protein called MamK in order to prevent simple diffusion of the

magnetosomes (Taoka *et al.*, 2017). These nanocrystals function as a permanent dipole which guides magnetotaxis (Faivre & Schüler, 2008).

Magnetotaxis generally has two manifestations: polar and axial magnetotaxis (Frankel *et al.*, 1997). Magnetotaxis towards only one pole is called “polar magnetotaxis” and is either north- or south-seeking; taxis along magnetic field lines without a specific affinity towards one pole is called “axial magnetotaxis” (Frankel *et al.*, 1997). MTB that exhibit axial magnetotaxis, such as many fresh water spirilla, perform frequent, spontaneous changes in direction (Frankel *et al.*, 1997) and use the magnetic field as an axis of motility along which MTB can travel, whereas bacteria performing polar magnetotaxis use the magnetic field to provide both an axis of motility and a direction to follow (Lefèvre *et al.*, 2014).

Because the magnetic field of the Earth is not very strong (0.25-0.65 gauss; Finlay *et al.*, 2010), I investigated how the spirillum (helical) MTB *Magnetospirillum magneticum* strain AMB-1 (here-on called “AMB-1”) responds to fields of various strengths induced by permanent bar magnets. In order to study this, I compared the movement of *Escherichia coli*, a coccoid (round) species not known to have any magnetotactic character, with that of AMB-1 (Zhang *et al.*, 2014). The overall aim of the study was to determine whether the MTB taxis would differ when exposed to fields of different strengths.

3 METHODS

3.1 Growth medium formulations and growth conditions

Luria broth (from here on, “LB”; LB Broth, Miller, Amresco®) was made at 2.5% vol. following manufacturer’s instructions. Magnetospirillum growth medium (from here on, “MSGM”) was made using the following protocol, which I modified from the vendor recommendation to avoid re-autoclaving pre-sterilized, potentially non-heat-stable reagents (ATCC® medium: 1653 Revised magnetic *Spirillum* growth medium (MSGM)). I added all powdered reagents to 1 L deionized water, adjusted the solution to a pH of 6.75 using solid NaOH pellets, autoclaved it at 121.1°C for 20 min, and cooled it to room temperature. Finally, I added Vitamin Supplement (ATCC® MD-VS) and Trace Mineral Supplement (ATCC® MD-TMS). The final MSGM contained, per liter of water: 10 mL vitamin solution, 5 mL mineral solution, 0.68 g (5mM) potassium phosphate, 0.12 g (1.4 mM) sodium nitrate, 0.035 g (200 µM) ascorbic acid, 0.37 g (2.5 mM) tartaric acid, 0.37 g (3.1 mM) succinic acid, 0.05 g (610 µM) sodium acetate, and 2 mL of 10 mM ferric quinate (20 µM final concentration) (Smith *et al.* 2006). MSGM was protected from photodegradation by wrapping in aluminum foil, and both LB and MSGM were stored at 3°C. MSGM was used in 15 mL aliquots in order to prevent accidental contamination of stock medium.

3.2 Inoculation and daily passaging of cultures.

I obtained *Escherichia coli*, strain DH5α (from here on “*E. coli*”) from Caitlyn Meiser (Rose-Hulman Institute of Technology, Chemical Engineering Department, Terre Haute, IN) and Irene Reizman, Ph.D. (Rose-Hulman Institute of Technology, Biology and Biomedical Engineering Department, Terre Haute, IN). I passaged *E. coli* daily by pulsing the previous day’s

culture with a vortex and transferring 2 μL to 1000 μL LB in a 1.5 mL centrifuge tube, then incubating the culture overnight at 30°C.

The MTB I used was *Magnetospirillum magneticum* strain AMB-1 (ATCC® 700264™), which I received as a frozen liquid culture. I inoculated initial cultures from frozen stock kept at -80°C by thawing the stock and adding 15 μL stock to 15 μL MSGM. Because AMB-1 seemed not to grow when inoculated at very low concentrations, I grew them by inoculating microcultures and gradually adding medium to increase volume. Over the course of two days, I added MSGM to double the volume of the sample until sample reached a total volume of 960 μL ; this was total of approximately three additions of MSGM, and then cultures were left to grow overnight in the 30°C incubator. For 2-5 days, the culture was centrifuged for 10 minutes at 3000 rpm and 50% of supernatant was replaced with fresh MSGM. After the culture grew to the point of turbidity, the percentage of medium replaced daily increased to 90-100%. After reaching turbidity, I passaged cultures once a week by adding 500 μL of culture to 500 μL of MSGM every week, while also replacing 1000 μL of MSGM every 1-2 days in order to maintain sufficient bacterial density for maximum visibility under the microscope.

3.3 Making stock cultures to be stored at -80°C

I prepared bacterial stock to be frozen and stored by centrifuging cultures and replacing supernatant medium with a solution that was one part sterile glycerol to four parts growth medium (LB for *E. coli* or MSGM for AMB-1) in a 1.5 mL microcentrifuge tube. The tube was then pulsed with a vortex and stored at -80°C.

3.4 Starting cultures from frozen stock

To use the frozen cultures, I thawed *E. coli* and passaged as per normal (see Section 3.2), as they proliferate quickly from a small inoculation quantity. To minimize loss of AMB-1 suspended in the glycerol-medium solution, I divided AMB-1 stock into three 500- μ L aliquots in 1.5 mL microcentrifuge tubes, diluted each with 700 μ L MSGM, and left them to incubate at 30°C for 12-24 hours. I then concentrated cultures via centrifuge for 10 min at 3000 rpm and fresh media was added, as per passaging procedures. This was repeated every 24-36 hr until a pellet was visible at the bottom of each tube (4-5 days), at which point I concentrated the cultures via centrifuge for 10 min at 3000 rpm and combined the contents of in order to maximize bacterial count.

3.5 Field strengths and magnetic field setup

I controlled magnetic field strengths by varying the distance between permanent bar magnets centered on the sample. The dimensions of each of the two magnets were 14.9 x 1.7 x 0.6 cm. I suspended the magnets directly above the microscope stage using three-prong clamps on ring stands so as not to weigh down the stage and disturb the focus of the microscope. Maarij Syed, Ph.D. (Rose-Hulman Institute of Technology, Physics and Optical Engineering Department, Terre Haute, IN) measured the strengths of the magnetic fields using an F. W. Bell Model 5180 gauss meter. Previous studies have used field strengths as strong as 700 gauss by using electromagnets in the form of Helmholtz coils (Zhao *et al.*, 2007). I chose the field strengths of 78 ± 4 gauss (2 cm between magnets) and 6.0 ± 1 gauss (10 cm between magnets) because of the ease of replication for future studies and because they fell in the range and strengths used in previous literature, both of which MTB are known to respond to. The distance between magnets

was verified at the start of each experiment using a standard 30-cm ruler. The strength of a field without magnets, meaning the MTB experienced only the ambient magnetic field of the Earth, was not measured, but it is known from literature to be 0.25-0.65 gauss (Finlay *et al.*, 2010).

3.6 Methods for viewing microbes

Microbes were viewed at 400X magnification, which was the highest available magnification that did not involve an oil immersion lens; the oil necessary for the immersion lens tended to stick to the cover slip and shift the sample out of frame. I used a Leica ICC50 W camera and the Leica AirLab app (Version 3.4) on a Leica DM750 compound microscope to take images. Images were taken at $t=0$ and $t=5$ minutes. In order to get as much of each sample in-frame as possible, I wanted to have the smallest possible sample volume; I determined qualitatively that 2 μL was my lower limit of reproducible pipetting (data not shown). Due to the magnification and sample size, I could not view the entire sample all at once, so I imaged each pole separately, using discrete samples in order to view each poles effect at initial exposure.

I viewed *E. coli* was viewed using the “hanging drop” method used in previous studies by placing a 2- μL drop of vortexed sample onto a cover slip and inverting it so the drop hangs off the underside of the glass (Mao *et al.*, 2014; Oestreicher *et al.*, 2012). I could then view the drop using a convex microscope slide. I also viewed both *E. coli* and AMB-1 were both viewed using a flat-bottomed well method, where I placed a 2- μL drop of sample in a 6-mm well on a 10-well microscope slide and covered it with a cover slip, so that the sample filled the entire well. Because my study was interested in net movement at the edges of the sample, which were closest to the magnets, images were take including the edge of the drop or well.

3.7 Viewing of microbes and magnetic field experiments

For each experiment, I randomly assigned the field strength and the order of observation of the magnetic north and south poles. I included the edge of the well in each image to capture any bacteria swimming into gaps in the edges of the wells and to observe any possible edge-clustering behaviors.

To view each sample, I pulsed the turbid cultures (*E. coli* or AMB-1) with a vortex and added a 2- μ L sample to a 6-mm well on a 10-well coated slide, followed by a cover slip. Previous studies have observed axial magnetotaxis in an observation period from 70 sec (Lefèvre *et al.*, 2014) to 14 min (Taoka *et al.*, 2017); 5 min was as a convenient observation period that was sufficiently long as to ensure movement of bacteria.

3.8 Manual counting of bacteria

Manual counting of bacteria was done using MATLAB to open each image, click on the each bacterium in the frame, and have MATLAB count the number of clicks (see APPENDIX A).

3.9 Automation of counting bacteria

Because manually counting bacteria can introduce sample-to-sample variability due to human error, it is logical to try to automate the process so that the treatment of each image is as similar as possible to avoid miscounting. In order to do this, I used MATLAB to convert the RGB images taken with the Leica ICC50 W to binary images consisting of only black and white pixels; this made the background white and the bacteria black. By counting the black pixels in an image, I could get a proxy for the biomass in each image and draw conclusions about net

bacterial movement using black-pixel counts as data. In addition, each image can be subdivided into sections and the black pixels counted for each section; this allows for discerning if bacteria tend to cluster in one area or another. (See APPENDIX B). Because the variable of interest was general change in biomass, rather than exact number of bacteria present at any given time, the black pixels were not converted back to number of bacteria.

3.10 Calculations and statistical analysis of data

Conclusions are drawn based on p-values from linear modeling. Net movement of bacteria was calculated by counting bacteria (both manually and autonomously) in images taken at the beginning and end of each experiment and subtracting the count at $t=5$ min from the count at $t=0$. Net movement percentage was calculated as the percentage of net movement of count at $t=0$. Statistical analysis was performed on using net movement; net movement percentage was calculated in order to normalize data to account for differences in initial bacteria in each experiment. All statistics were performed using R (Version 3.4.1). (See APPENDIX C).

4 RESULTS

4.1 Images were taken using the flat-bottomed well technique for reproducibility of depth.

Images taken of *E. coli* using the hanging drop method had high contrast, but it was difficult to reproducibly focus on a depth below the cover slip, to which dead or immobile bacteria had a tendency to stick and against which few motile bacteria were observed (full data not shown; see APPENDIX E for an example). Images taken with the flat-bottomed well method had lower contrast bacteria, but the size of the area in focus was more reproducible, as the cylindrical shape of the well lent itself to reproducible depth of focus. Therefore, images for the remainder of experiments were taken using the flat-bottomed well technique.

4.2 Manual and autonomous counting methods did not agree statistically.

Flat-bottomed well images of *E. coli* and AMB-1 were counted manually (see APPENDIX A), and autonomously (see APPENDIX B). Because the variable of interest was net bacterial movement rather than number of bacteria in each frame, the number of black pixels counted were analyzed without alteration, rather than converting the counted pixels into number of bacteria. Manual and autonomous counting did not agree for the statistical significance of magnetic pole of observation ($p_{\text{manual}}=0.27483$; $p_{\text{autonomous}}=0.0287$) or of species ($p_{\text{manual}}=0.00596$; $p=0.6878$) (see Figures 1 and 2). Neither counting protocol indicated significance of either 2-cm separation (78 ± 4 gauss; $p_{\text{manual}}=0.93272$; $p_{\text{autonomous}}=0.9513$) or 10-cm separation (6.0 ± 1 gauss; $p_{\text{manual}}=0.05476$; $p_{\text{autonomous}}=0.1515$) (see Figure 1 and 2).

4.3 Magnetic field strengths tested had no significant effect on net movement of bacteria.

I took images of *E. coli* and AMB-1 at each pole on opposite ends of the flat, 6-mm well before and after five-minute exposure to either the ambient geomagnetic field of 0.25-0.65 gauss (no additional magnets; Finlay *et al.*, 2010), 78 ± 4 gauss (2-cm separation between magnets), or 6.0 ± 1 gauss (10-cm separation between magnets). Net movement was calculated by manually counting the bacteria in each image and subtracting bacteria at $t=5$ min from $t=0$ (Figure 1 shows net movement percentage, which is net movement as percent of count at $t=0$ in order to normalize for differences between experiments in bacteria at $t=0$). Linear modeling on manually counted data showed a statistically significant effect ($p < 0.05$) of the date on which the experiment was performed (data not shown), so that variable was omitted for the remainder of the analysis. Manual counting revealed that species ($p=0.00596$) and initial count at $t=0$ ($p=0.04086$) had significant effects on net movement, but pole of observation ($p=0.27483$), 2-cm separation between magnets (field strength 78 ± 4 gauss; $p=0.93272$), and 10-cm separation between magnets (field strength 6.0 ± 1 gauss; $p=0.05476$) had no statistically significant effect on net movement of bacteria (Figure 1).

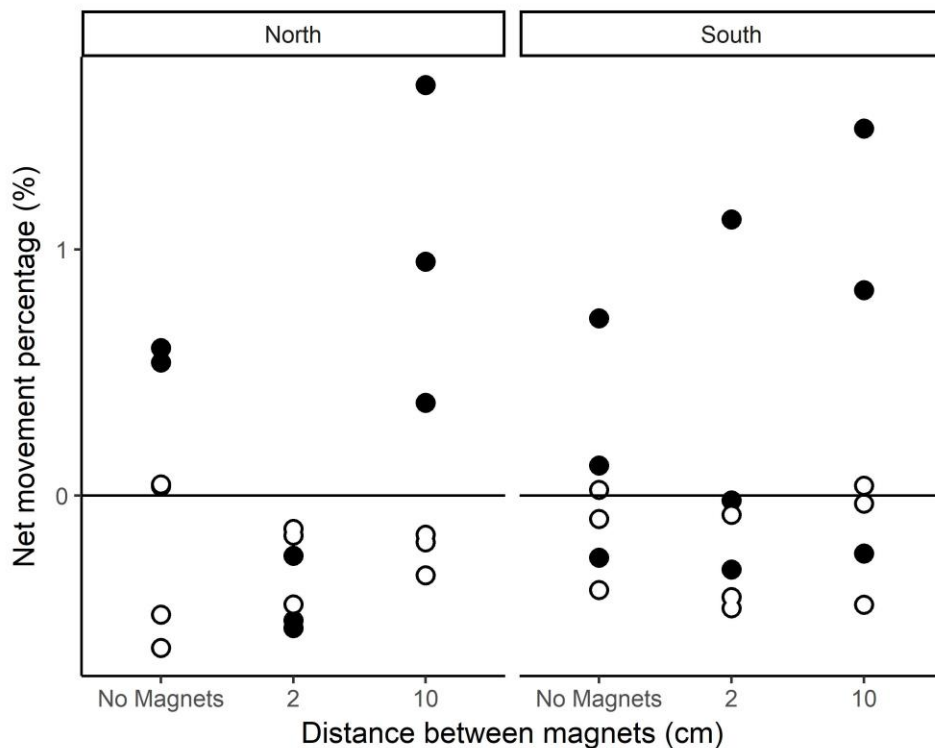


Figure 1. AMB-1 (filled circle) and *E. coli* (hollow circle) were manually counted at $t=0$ and $t=5$ min on the north and south sides of the sample under varying field strengths controlled by distance the between bar magnets (no magnets, 0.25-0.65 gauss; 2-cm separation, 78 ± 4 gauss; 10-cm separation 6.0 ± 1 gauss) Each data shown is an independent experiment. (See APPENDIX A for MATLAB code; see APPENDIX D for data).

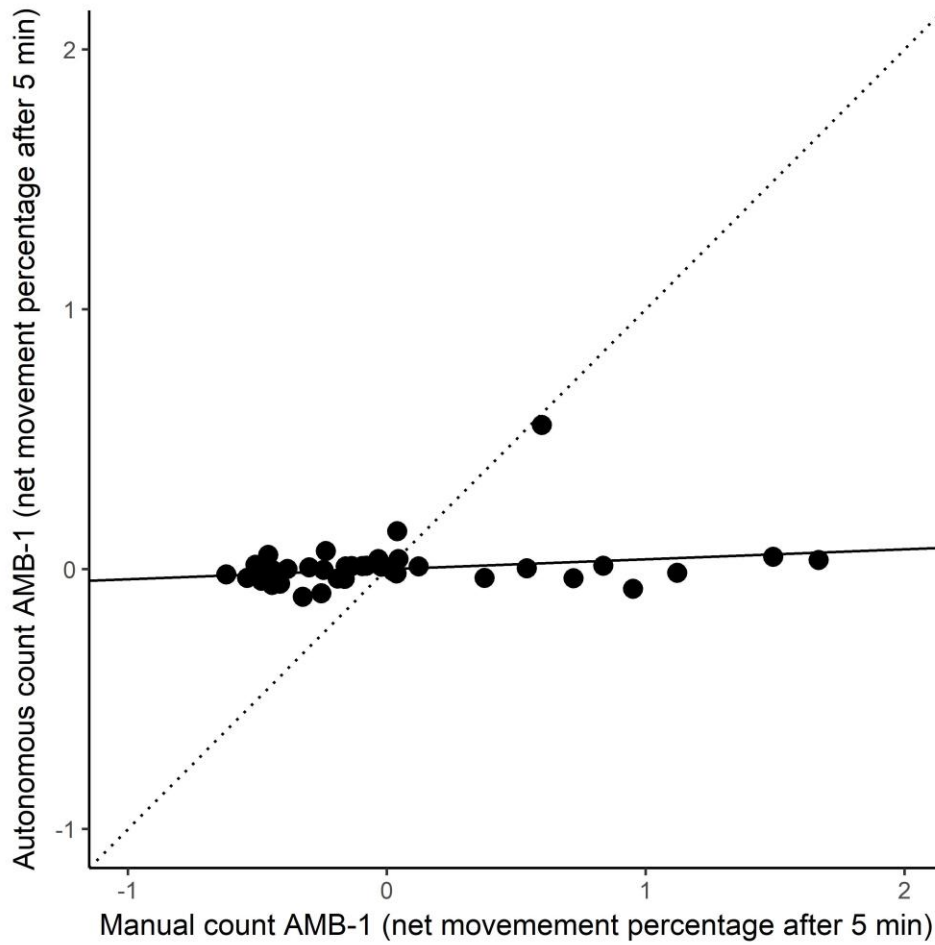


Figure 2. AMB-1 was counted manually and autonomously, and percentage change after 5 min is plotted to show how the two counting methods compared. The dotted line has a slope of 1 and models identical counting. A linear regression (solid line) of autonomous versus manual counts had a slope of 0.03836.

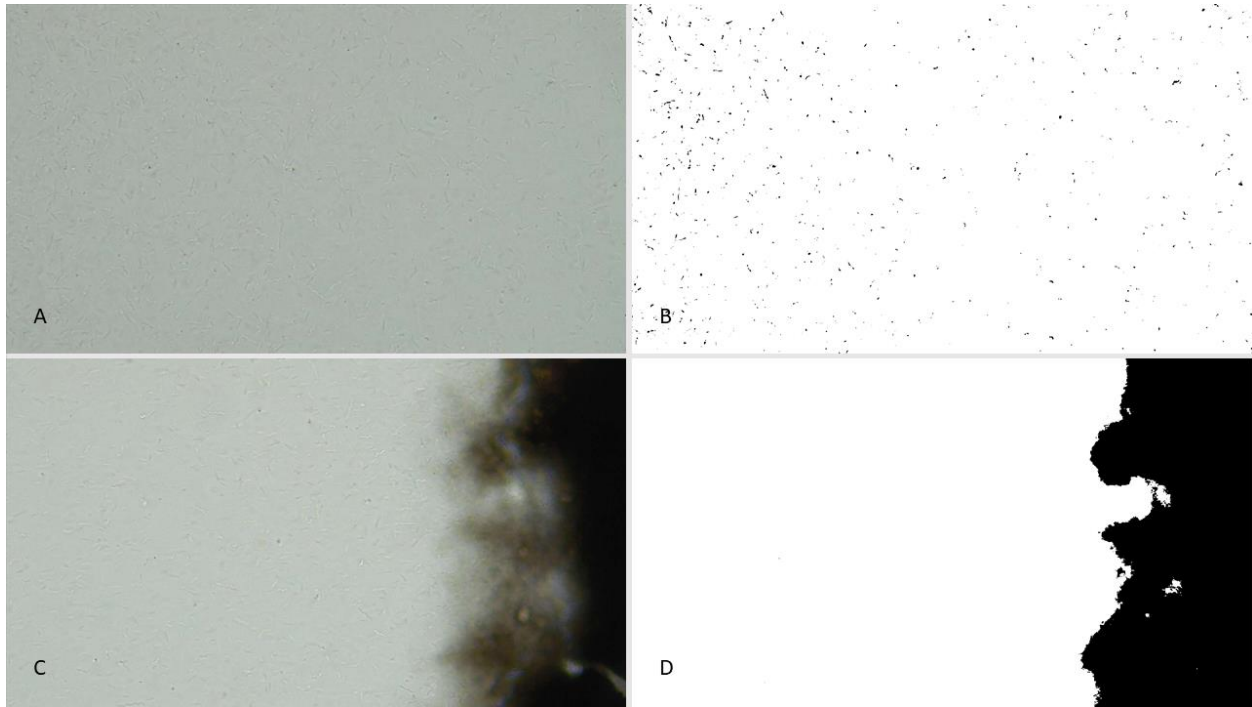


Figure 3. Raw image (A, C) was counted manually (see APPENDIX A); image was then made binary and counted autonomously (respectively, B, D; see APPENDIX B). In order to capture bacteria on edge of well, images after Image Number 613 (A, B) included image of edge of well (i.e. Image Number 614; C, D). A and C have similar numbers of bacteria present. B shows significantly more bacteria detected by the autonomous counting program than in D because A is higher contrast than C.

5 DISCUSSION

5.1 Axial magnetotaxis is unaffected by field strength.

Magnetotaxis has been categorized into polar magnetotaxis, which uses a magnetic field to guide both the axis of motility and the preferential direction of travel, and axial magnetotaxis, which uses a magnetic field for the axis of motility but does not favor one pole over the other (Frankel *et al.*, 1997). One organism which is known to exhibit axial magnetotaxis is *Magnetospirillum magneticum*, and so I used *M. magneticum* strain AMB-1 as my representative organism (Bazylinski *et al.*, 2004; Taoka *et al.*, 2017). The aim of this study was to investigate whether axial magnetotaxis changes with varied field strengths. The strength of the ambient geomagnetic field is 0.25-0.65 gauss (Finlay *et al.*, 2010), and previous literature has used fields of strengths as high as 700 gauss (Zhao *et al.*, 2007). Observation periods in previous studies ranged from 70 sec (Lefèvre *et al.*, 2014) to 14 min (Taoka *et al.*, 2017). This study used intermediate field strengths of 78 ± 4 gauss and 6.0 ± 1 gauss, and found no statistical difference in net movement after five minutes in axial magnetotactic behaviors. While it has been suggested in previous studies that axial magnetotaxis is not affected flipping magnetic poles by 180° (Bazylinski *et al.*, 2004), the results of this study suggest that axial magnetotaxis is also unaffected by changes in field strength. This conclusion would allow future studies of axial magnetotaxis to be performed using fields of relatively low strength, which has the potential to reduce the monetary cost and physical space of future research on axial magnetotaxis.

5.2 The experimental setup yielded results in agreement with previous studies.

One of the benefits of significant consequences of this study using magnetic fields generated by permanent magnets is its agreement with previous studies which used

electromagnets. The difference between net movement of *E. coli* and AMB-1 indicate that AMB-1 is magnetotactic, while *E. coli* is not, as expected (Zhang *et al.*, 2014). The similar response of AMB-1 to magnetic fields at the north and south poles suggests axial magnetotaxis, which also agrees with previous studies (Frankel *et al.*, 1997; Lefèvre *et al.*, 2014; Taoka *et al.*, 2017; Zhao *et al.*, 2007). Previous studies used Helmholtz coils to provide precise, uniform electromagnet-generated fields (Lefèvre *et al.*, 2014; Mao *et al.*, 2014; Zhao *et al.*, 2007). However, Helmholtz coils require an outside power source and take up considerable amounts of space in comparison to a bar magnet; to generate a 0.002-gauss field may require coils of radius 20 cm (Beiranvand, 2017; Trout, 1988); by contrast, the dimensions of each of the bar magnets used in this study were 14.9 x 1.7 x 0.6 cm. The considerably smaller amount of space needed for this study to similarly conclude axial magnetotaxis in AMB-1 presents the possibility of down-sizing future experiments, which would make the experiments more broadly accessible.

5.3 Both hanging drop and flat-bottomed well method presented challenges.

The hanging drop method of viewing used in previous studies (Mao *et al.*, 2014; Oestreicher *et al.*, 2012) provides the advantage of high-contrast images (see Section 5.4). However, it has significant disadvantages of large surface area available for evaporation and the shrinking width of the sample as the focus descends the meniscus, which is difficult to replicate. Some of these issues are addressed by using the flat-bottomed well method: the width of the sample at any given height is relatively similar because the well is cylindrical, rather than a meniscus, and there is less surface area open to evaporation because the sample fills the entire inner surface area of the well and is enclosed by the cover slip, so there is less air for the sample to evaporate into. However, because the study investigated bacterial collecting on the edge of the

sample (drop or well) over time, it was necessary to have the edge of the sample in each frame, which made bacteria in the flat-bottomed well images very low-contrast due to the color of the slide material (see Section 5.4). Therefore, while the flat-bottomed well method was selected because of the reproducibility of focusing afforded by the cylindrical shape of the wells, autonomous counting was not possible using this method.

5.4 Autonomous counting was unable to count flat-bottomed well images.

While the autonomous counting program was able to count the bacteria viewed using the hanging drop method, it was unable to differentiate between the bacteria and the background in images of the flat-bottomed well method. All images were taken including the edge of the sample in order to capture any grouping on the edge of the sample which might have occurred. The background outside the edge of the hanging drop samples was a similar lightness value as the background of the sample, and so the microscope was able to auto-shift the brightness to put the bacteria in the highest possible contrast. However, the material of the wells in the flat-bottomed well method is black, meaning the background outside the sample was a large, dark area which caused the microscope's auto-brightness to compensate by making adjusting the contrast, and consequently, the bacteria were low-contrast from their background. Because the bacteria had insufficient contrast in the flat-bottomed well method, the autonomous counting program was unable to differentiate bacteria from background, and therefore the statistics drawn from the autonomously counted data differed from the manually counted data. However, autonomous counting was able to quickly count the black pixels (proxy for biomass) a large quantity of images (see APPENDIX E), and has the potential to be helpful in future studies using high-contrast images.

5.5 Limitations of this study

Although statistical significance has been drawn from linear modeling, it should be noted that the maximum change is only 1.7%. Additionally, Helmholtz coils allow for greater precision in controlling field strength, and multiple orthogonal pairs of coils can achieve a very uniform field (Mao *et al.*, 2014) that would be difficult to achieve using bar magnets.

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7 APPENDIX A: Using MATLAB to manually count bacteria

Below is the MATLAB code used to record manual counting of microbes.

```
% MDP_Final_00_ClickCount.m

% Purpose: pull up original image; click the bacteria and it will count the
% clicks to count the bacteria in the image.

% VARIABLES
% (0) outputPath: the path to the folder where output file will be saved
% (1) file1: file name of image navigated to (original image)
% (1) path1: path to file of navigated to (original image)
% (1) imageSelected: combination of path1 and file1
% (1) origImage: image that you're clicking bacteria for
% (2) bactPoints: matrix with coordinates of every point clicked
% (3) rows: number of rows (points) you clicked
% (3) cols: number of columns in the matrix [rows, cols]; not going to use this
% variable
% (3) endMessage: end message for when done clicking
% (3) outputName: name for output .csv file

%% 0. Clear workspace and SET OUTPUT PATH

clear
clc

outputPath = 'C:\Users\pascomd\Documents\00 Senior Thesis\thesis-work\outputs\2018-01-19 E coli
Distance';

%% 1. Grab original image and overlay grid.

% Pick image
[file1, path1] = uigetfile('*.jpeg', 'Select Image for Processing');
imageSelected = fullfile(path1, file1);
origImage = imread(imageSelected);

% Overlay grid
imshow(origImage) % show image
hold on % keep image instead of replacing it

M = size(origImage,1);
N = size(origImage,2);

for k = 1:200:M % sets up 200-pixel grid w/ 2-color lines for contrast
    x = [1 N];
    y = [k k];
    plot(x,y,'Color','w','LineStyle','-');
    plot(x,y,'Color','k','LineStyle',':');
end

for k = 1:200:N % sets up 200-pixel grid w/ 2-color lines for contrast
    x = [k k];
    y = [1 M];
    plot(x,y,'Color','w','LineStyle','-');
    plot(x,y,'Color','k','LineStyle',':');
end

hold off

%% 2. Click bacteria in image. Hit 'Enter' to finish clicking.

waitfor(msgbox('Click the bacteria. Hit ENTER to exit to finish.'))
bactPoints = ginput;
close(figure(1))

%% 3. Print and output result as a text file.

[rows, cols] = size(bactPoints);
```

```
% Print
endMessage = sprintf('You clicked %g bacteria in this image.', rows);
msgbox(endMessage)

% Name output file
outputMatrix = [rows, cols];
outputName = char( inputdlg('What will be the name of the output files (.csv)?'));

% Save data
writetable( array2table(outputMatrix), fullfile(outputPath, strcat(outputName, '.csv')));
```

8 APPENDIX B: Using MATLAB to autonomously count bacteria

Below is the MATLAB code used to autonomously count bacteria in an image by converting images of bacteria to binary images and counting the black pixels.

8.1 Unique functions

```
function [section_length] = imgSectLength( image, section_number, section_direction )
% imgSectLength Gives length of section, given image, number of sections,
% and direction of divisions
% Outputs length of section when given image, number of sections, and
% direction of divisions. 'sect_num' must be greater than 0, and
% `sect_dir` can be 'horz' (2) or 'vert' (1). If not 'horz' (2), assumed to be
% 'vert' (1).

% `size(img)` yields [height, width]

image_size = size(image);

% 1. Set dimension to slice
if section_direction == 2 % 1 is 'Vertical'; 2 is 'Horizontal'
    image_dimension = image_size(1);
else
    image_dimension = image_size(2);
end

% 2. Divide slicing dimension by number of slices and output section_length
section_length = image_dimension / section_number;

end
```

8.2 Single-image processing

```
% MDP_Final_01_SingleImage.m

% Purpose: to convert an image to binary and count the number of black
% pixels in the binary image. This script is to be called by
% "MDP_Final_02_MultipleImages.m"

% Variables:
% (0) files: file name of image
% (0) paths: path to image file
% (0) sect_num: number of sections desired for image
% (0) sect_dir: direction of sections (1 for 'vertical' or 2 for 'horizontal')
% (0) question_seeLines: determines whether would like to see sectioned
% images
% (1.1) img_selected: full path and file name to image file
% (1.1) img_orig: image file, unaltered
% (1.3) grey_thresh: threshold value to decide what is white versus black
% (1.4) img_binary: binary image
% (2.1) img_size: [height, width] of the image
% (2.1) sect_length: length of section
% (2.2) line_top: top of line for vert sections
% (2.2) line_bot: bottom of line for vert sections
% (2.2) line_left: left of line for horz sections
% (2.2) line_right: right of line for horz sections
% (2.3) sect_start: array containing placement of lines as per section length
% (2.3) sect_end: array containing placement of end of sections
% (3) array_white: array of white pixels of sections
% (3) array_total: array of total pixels of sections
% (3) array_black: array of black pixels in sections
```

```

%% 0. Information to be input by another script
% This information will be commented out under normal circumstances, but
% remains for debugging purposes.

%files = 'Image000.jpeg'
%paths = 'C:\Users\pascomd\Documents\00 Senior Thesis\thesis-work\photo-video\2017-12-21 AMB-1
Distance\'
%question_sect = 1 % 1 is 'Yes'; 2 is 'No'
%sect_num = 5
%sect_dir = 'NA' % 1 is 'Vertical'; 2 is 'Horizontal'; 'NA' is no sections
%question_saveImages = 1 % 1 is 'Yes'; 2 is 'No'
%question_seeLines = 1 % 1 is yes; 2 is no

%% 1. Convert image to binary.

% 1.1 Import image using 'files' and 'paths'
% img_selected = fullfile(paths, files); % for debugging purposes only
% img_orig = imread(img_selected); % for debugging purposes only

% 1.2 View image
% imshow(img_orig) % for debugging purposes only

% 1.3 Set threshold for black versus white
grey_thresh = graythresh(img_orig);

% 1.4 Convert image to binary by toggling threshold level
img_binary = im2bw(img_orig, 0.93*grey_thresh); % often 0.93*grey_thresh

%% 2. Divide image into desired sections
% Formatting:
% line([x1,x2],[y1,y2])

% 2.1 Determine size of sections
img_size = size(img_binary);
sect_length = imgSectLength(img_binary, sect_num, sect_dir);

% 2.2 Determine line starting points based on image size
switch sect_dir
    case 1 % vert sections have y1 = y2
        line_top = 1;
        line_bot = img_size(1);
    case 2 % horz sections have x1 = x2
        line_left = 1;
        line_right = img_size(2);
end

% 2.3 Determine line ending points based on section lengths
for k = 1:sect_num
    sect_start(k) = (k-1) * sect_length + 1;
end

for k = 1:sect_num
    sect_end(k) = k * sect_length;
end

%% 3. Create sections and count the number of black pixels in each section
% Formatting:
% new_sect = Array(Top:Bottom, Start:End)
% Array(rows, columns)

switch sect_dir
    case 1 % 'Vertical' is 1
        % White Pixels (value = 1)
        for k = 1:sect_num
            array_white(k) = sum( sum( img_binary(line_top:line_bot, sect_start(k):sect_end(k))
));
        end

        % Total Pixels (height of section * width of section + 1 because
        % sections are inclusive)
        for k = 1:sect_num

```

```

        array_total(k) = line_bot * (sect_end(k) - sect_start(k) + 1);
    end

    % Black Pixels (total pixels - white pixels = black pixels)
    array_black = array_total - array_white;

    case 2 % 'Horizontal' is 2
        % White Pixels (value = 1)
        for k = 1:sect_num
            array_white(k) = sum(sum(img_binary(sect_start(k):sect_end(k),
line_left:line_right)));
        end

        % Total Pixels(height of section * width of section + 1 because
% sections are inclusive)
        for k = 1:sect_num
            array_total(k) = (sect_end(k) - sect_start(k) + 1) * line_right;
        end

        % Black Pixels(total pixels - white pixels = black pixels)
        array_black = array_total - array_white;

    case 'NA' % No sections desired
        % White Pixels (value = 1)
        array_white = sum( sum( img_binary));

        % Total Pixels (height of image * width of image + 1 because
% inclusive)
        array_total = img_size(1) * img_size(2) + 1;

        % Black Pixels (total pixels - white pixels = black pixels)
        array_black = array_total - array_white;

end

%% 4. Draw lines
% Formatting:
% line([x1,x2],[y1,y2])

% Only view and save and annotate images if the viewer wants to see them (no reason to do that
% computing if we don't need to)
if question_saveImages == 2 % 2 is no
    % Kick out of this script if don't want to save images
    return
else
    imshow(img_binary)

    % Only annotate images if user wants to
    if question_seeLines == 2 % 2 means no
        return
    else
        hold on
        for k = 1:sect_num
            switch sect_dir
                case 1 % 'Vertical'
                    line([sect_start(k), sect_start(k)], [line_top, line_bot])
                case 2 % 'Horizontal'
                    line([line_left, line_right], [sect_start(k), sect_start(k)])
            end
        end
    end
end
end
end

```

8.3 Multiple-image processing

```

% MDP_Final_02_AutoCount_MultipleImages.m

% Purpose: to count the black pixels in multiple images by running
% 'MDP_Final_01_AutoCount_SingleImage.m'

% Variables:
% (1.1) question_fileNum: determines whether we're getting multiple files
% (1.1) files: file names for images to be analyzed
% (1.1) paths: path to each file for image to be analyzed
% (1.2) output_path: path to which files will be saved
% (1.2) output_name: name of output file
% (1.3) question_sect: determines whether will use sections
% (1.4) sect_num: number of sections (1 means not sectioning)
% (1.4) sect_dir: direction of sections
% (1.5) question_saveImages: ask whether would like to save edited images
% (1.6) question_seeLines: ask whether would like to see lines on edited
%       image
% (2.1) fileNum: number of files we're dealing with
% (2.1) i: index
% (2.2) img_selected: full path and file name to image file
% (2.2) img_orig: image file, unaltered
% (2.3) img_num: image number (for the sake of proper labeling)
% (2.3) fileCurrent: indexed file so I can grab the image number
% (2.4) output_pngname: actual name of image to be output plus .png extension
% (2.5) answer_blackcount: number of black pixels in each section of each
%       image selected for analysis

%% 1. Clear workspace and ask user for the images to be processed

clear
clc

% 1.1 Select image(s) to be processed
question_fileNum = menu('How many files are you selecting?',...
    'One', 'Many'); % 1 is 'One'; 2 is 'Many'
if question_fileNum == 2
    [files, paths] = uigetfile('*.jpeg', 'Select Image(s) for Processing', 'MultiSelect', 'on');
else
    [files, paths] = uigetfile('*.jpeg', 'Select Image(s) for Processing');
end

% 1.2 Select output path and name of file
output_path = uigetdir('C:\Users\pascomd\Documents\00 Senior Thesis\thesis-work\',...
    'Please select the folder where you would like place outputs. ');
output_name = char( inputdlg('What would you like to call your output file(s)?') );

% 1.3 Would you like to section your images?
% Output: 'Yes' is 1; 'No' is 2
question_sect = menu('Would you like to use multiple sections?',...
    'Yes', 'No');

% 1.4 How many sections? What direction?
switch question_sect
    case 1 % if user clicked 'Yes'
        sect_num = str2num( char( inputdlg('How many sections do you want?') ) );
        sect_dir = menu('What direction would you like the sections?',...
            'Vertical', 'Horizontal'); % Output: 'Vertical' is 1; 'Horizontal' is 2
    case 2 % if user clicked 'No'
        sect_num = 1; % If no sections desired, will ignore sections
        sect_dir = 'NA'; % If no sections desired, will ignore sections
end

% 1.5 Would you like to view and save processed images with section lines?
question_saveImages = menu('Would you like to save your processed images?',...
    'Yes', 'No'); % Output: 'Yes' is 1; 'No' is 2

% 1.6 Only ask if would like to see section lines if BOTH: asked to save images and asked to
section images

```



```

if question_saveImages == 1
    if question_sect == 1
        question_seeLines = menu('Would you like to annotate images with section lines?',...
            'Yes', 'No'); % Output: 'Yes' is 1; 'No' is 2
    else
        question_seeLines = 2; % default is 2
    end
end
end

%% 2. Use user information to process all images

% 2.1 Go through each file. If only one file selected, set size(files, 2)
% to 1

switch question_fileNum
    case 1 % 1 is 'One' file selected
        fileNum = 1;
    case 2 % 2 is 'Many' files selected
        fileNum = size(files, 2); % for one file, this counts the number of characters in file
        name, which is not what we want
    end

for i = 1:fileNum % range is 1 to 1 OR 1 to length of 'files' vector, depending on number of
files selected
    % 2.2 Differentiate between vector of image titles vs. characters in
    % one image's file name
    if question_fileNum == 1 % 'One' file selected
        img_selected = char( fullfile(paths, files) );
        img_orig = imread(img_selected);
    else % 'Many' files selected
        img_selected = char( fullfile(paths, files(i)) ); % read path name as 'char'
        img_orig = imread(img_selected);
    end

    MDP_Final_01_AutoCount_SingleImage

    % 2.3 Determine image number for record-keeping purposes
    if question_fileNum == 1
        img_num = files(6:8); % characters 6 to 8 of 'ImgXXX.jpg'
    else
        fileCurrent = char( files(i));
        img_num = fileCurrent(6:8);
    end

    % 2.4 Save images to output path
    output_pngname = sprintf( strcat(output_name, ' AnnotatedImage%d.png'), str2num(img_num));
    saveas(gcf, fullfile(output_path, output_pngname), 'png')

    % 2.5 If first time through the loop, start the array containing the
    % answer (black pixel count). If not, add the new `black_array`
    % to the bottom of the array `answer_blackcount`.
    if i == 1
        answer_blackcount = array_black;
    else
        answer_blackcount = cat(1, answer_blackcount, array_black);
    end
end

%% 3. Export black pixel data to where
writetable( array2table(answer_blackcount), fullfile(output_path, strcat(output_name, '.xlsx')));

```

9 APPENDIX C: Using R to perform statistical analysis

Below is the R code used to generate plots and perform statistical analysis.

```
# MDP_R-Analysis.R
# To use the bacteria counted manually and autonomously and produce data visualization figures.

### FILL OUT THIS PART
# Set Correct File Names
filename_manualData <- 'outputs/DistanceData_ManualCount.xlsx'
filename_autoData <- 'outputs/DistanceData_AutoCount.xlsx'

# 1. Libraries:
library('ggplot2', lib.loc='~/R/R-3.4.1/library')
library('readxl', lib.loc='C:/R/library')

### 2. Read Data
# 2.1 Manual data 'StartBac' and 'EndBac' units are counted bacteria; Auto data 'StartBac' and
# 'EndBac' are black pixels
data_manual <- read_excel(filename_manualData, sheet = 2)
data_auto <- read_excel(filename_autoData)

# 2.2 Combine into one large dataframe with all information
data_total <- data_manual
data_total$StartPix <- data_auto$StartBac
data_total$EndPix <- data_auto$EndBac

# 2.3 Take difference between starting and ending bacteria ('bac') and pixels ('pix')
data_total$DiffBac <- data_total$EndBac - data_total$StartBac
data_total$DiffPix <- data_total$EndPix - data_total$StartPix

# 2.4 Show percentages
data_total$PercentDiffBac <- data_total$DiffBac / data_total$StartBac
data_total$PercentDiffPix <- data_total$DiffPix / data_total$StartPix

# 2.5 Change distance of 0 to 'No Magnets' for clarity of reading
data_total$Distance[data_total$Distance == 0] <- 'No Magnets'

# 2.6 Rearrange levels of labels for plots later (order should be: 'No Magnets', '2', '10')
data_total$Distance <- as.factor(data_total$Distance)
data_total$Distance <- factor(data_total$Distance, levels=rev(levels(data_total$Distance)))

## 3. Plotting MANUAL data visualizations and re-doing based on statistics

# 3.1 Presets for how the data should look
theme_set(theme_bw())

# 3.2 MANUAL COUNT data visualization and linear model (testing StartBac, Distance, Pole,
# Species, Date). E. coli are hollow shapes; color as.factor(Date)
plot_manual <- ggplot(data_total, aes(x = as.factor(Distance), y = PercentDiffBac, color =
as.factor(Date))) +
  theme_classic() +
  geom_point(size = 3) +
  geom_point(data = data_total[data_total$Species == 'Ecoli',], size = 2, color = 'white') +
  xlab('Distance Between Magnetic Poles (cm)') +
  ylab('Percentage Change in Bacteria after 5 min (%)') +
  geom_hline(yintercept = 0) +
  facet_grid(~Pole)
plot_manual

model_manual <- lm(data = data_total, DiffBac ~ StartBac + as.factor(Distance) + Pole + Species +
Date)
summary(model_manual)
# INTERPRETATION: Date definitely doesn't seem to play any role (p>0.1) so we'll exclude that and
remove the color as.factor(Date) since expect that not to have effect and it doesn't

# 3.3 MANUAL COUNT data visualization and linear model (testing StartBac, Distance, pole,
# Species)
```

```

plot_manual <- ggplot(data_total, aes(x = as.factor(Distance), y = PercentDiffBac)) +
  theme_classic() +
  geom_point(size = 3) +
  geom_point(data = data_total[data_total$Species == 'Ecoli',], size = 2, color = 'white') +
  xlab('Distance between magnets (cm)') +
  ylab('Net movement percentage (%)') +
  geom_hline(yintercept = 0) +
  facet_grid(~Pole)
plot_manual

model_manual <- lm(data = data_total, DiffBac ~ StartBac + as.factor(Distance) + Pole + Species)
summary(model_manual)

# INTERPRETATION: StartBac (p<0.05) and Species (p<0.05) both make a difference; Pole (p>0.05)
and Distance do not (p>0.05)
# DISCUSSION:
# Date has no effect, which is good
# Pole has no effect so probably axial magnetotaxis (different from observed, but
Magnetospirillum are usually axial)
# Species has effect, so E. coli is different from AMB-1 (this is expected)
# Distance has no effect, so potentially not different enough fields, but also if can follow
Earth's field, can probs deal with very weak fields; not high enough field at 2cm?
# StartBac has effect, so something is going on there but I don't know what that makes seeding
number relevant

# 4. Plot Autonomous vs. Manual

# 4.1 Plotting comparison of autonomous and manual counts with a line over x=y to show deviation
plot_both <- ggplot(data_total, aes(x = PercentDiffBac, y = PercentDiffPix)) +
  theme_classic() +
  geom_point(size = 3) +
  geom_abline(slope = 1, intercept = 0, linetype = "dotted") +
  scale_x_continuous(limits = c(-1, 2)) +
  scale_y_continuous(limits = c(-1, 2)) +
  ylab('Autonomous count AMB-1 (net movement percentage after 5 min)') +
  xlab('Manual count AMB-1 (net movement percentage after 5 min)')
plot_both

# 4.2 Linear regression to see how different the slo
test <- lm(data = data_total, PercentDiffPix ~ PercentDiffBac)
summary(test)

# 4.3 Add a regression line with the
plot_both <- plot_both + geom_abline(slope = 0.03836)
plot_both

# 5. Export those pretty plots!
output_path <- 'outputs/'
ggsave(paste(output_path, 'plot_manualCount.jpg', sep = ''), plot = plot_manual, width = 5,
height = 4, units = 'in', dpi = 600)
ggsave(paste(output_path, 'plot_comparisson.jpg', sep = ''), plot = plot_both, width = 5, height
= 5, units = 'in', dpi = 600)

## 4. Plotting AUTONOMOUS COUNT data visualization and linear model (testing StartBac, Distance,
Pole, Species, Date). E. coli are hollow shapes; color as.factor(Date)

# 4.1 AUTO COUNT data visualization and linear model (testing StartPix, Distance, Pole, Species,
Date). E. coli are hollow shapes; color as.factor(Date)
plot_auto <- ggplot(data_total, aes(x = as.factor(Distance), y = PercentDiffPix, color =
as.factor(Date))) +
  theme_classic() +
  geom_point(size = 3) +
  geom_point(data = data_total[data_total$Species == 'Ecoli',], size = 2, color = 'white') +
  xlab('Distance Between Magnetic Poles (cm)') +
  ylab('Percentage Change in Pixelx after 5 min (%)') +
  geom_hline(yintercept = 0) +
  facet_grid(~Pole)
plot_auto

```

```

model_auto <- lm(data = data_total, DiffPix ~ StartPix + as.factor(Distance) + Pole + Species +
Date)
summary(model_auto)
# INTERPRETATION: Date definitely doesn't seem to play any role (p>0.1) so we'll exclude that and
remove the color as.factor(Date) since expect that not to have effect and it doesn't

# 4.2 AUTO COUNT data visualization and linear model (testing StartBac, Distance, pole, Species)
plot_auto <- ggplot(data_total, aes(x = as.factor(Distance), y = PercentDiffPix)) +
  theme_classic() +
  geom_point(size = 3) +
  geom_point(data = data_total[data_total$Species == 'Ecoli',], size = 2, color = 'white') +
  xlab('Distance Between Magnetic Poles (cm)') +
  ylab('Percentage Change in Pixelx after 5 min (%)') +
  geom_hline(yintercept = 0) +
  facet_grid(~Pole)
plot_auto

model_auto <- lm(data = data_total, DiffPix ~ StartPix + as.factor(Distance) + Pole + Species)
summary(model_auto)
# INTERPRETATION: StartPix (p<0.05), and Pole (p<0.05) have effect; Distance (p>0.05) and Species
(p>0.05) do not
# DISCUSSION:
# StartPix has an effect; similar to MANUAL COUNT, I'm not sure what to do with this
# Pole has an effect, which is exciting; this remains if I remove the super high point that is
'2017-12-17 No Magnets AMB-1', which is kinda cool
# Distance has no effect; see MANUAL COUNT's DISCUSSION
# Species has no effect, indicating no difference between MTB and non-magnetotactic bacteria
under this analysis

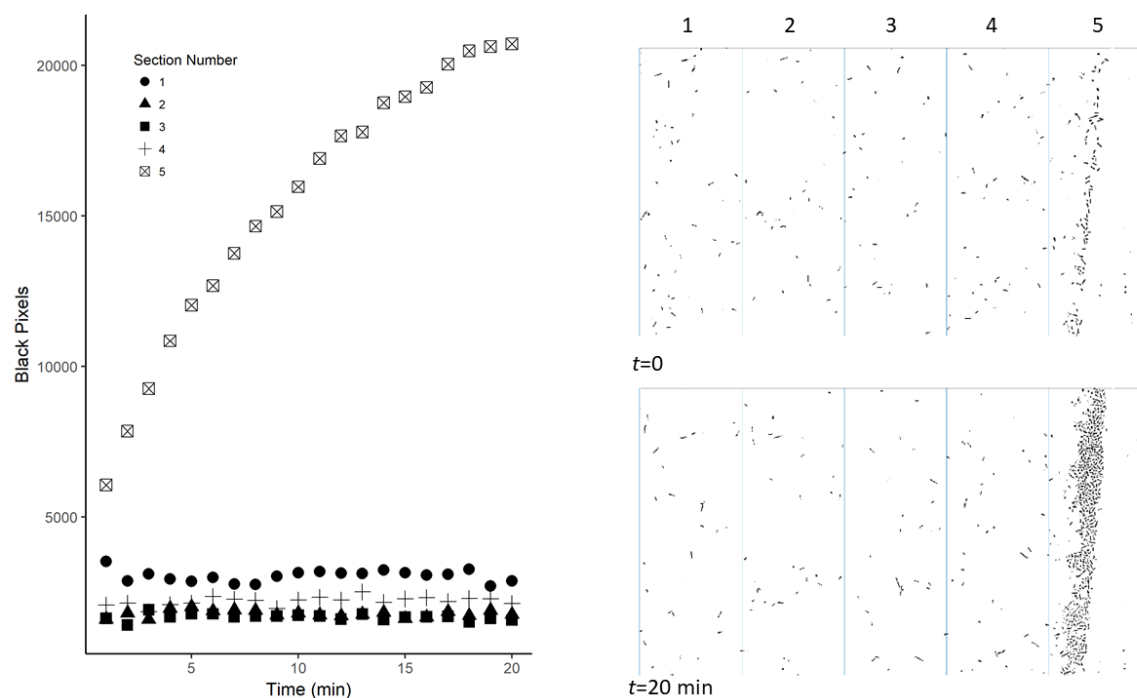
```

10 APPENDIX D: Experimental data from manual and autonomous counting

Below is all data visualized in *Figure 1* and *Figure 2*. “Date” is the date on which each experiment was performed; “Species” is species in question. “Distance” is distance between bar magnets (cm). “Pole” is magnetic pole at which experiment was performed; “StartImage” is the Image Number for $t = 0$; “EndImage” is the Image Number for $t = 5$; “StartBac” and “EndBac” are the number of bacteria manually counted in “StartImage” using MATLAB code found in APPENDIX A; “StartPix” and “EndPix” are the number of black pixels autonomously counted in “StartImage” and “EndImage” using MATLAB code found in APPENDIX B. Each row is an independent experiment. The software’s maximum image number was 999; any further images were counted starting back at Image No. 001.

<u>Date</u>	<u>Species</u>	<u>Distance</u>	<u>Pole</u>	<u>StartImage</u>	<u>EndImage</u>	<u>StartBac</u>	<u>EndBac</u>	<u>StartPix</u>	<u>EndPix</u>
12/17/2017	AMB-1	No magnets	South	551	581	87	65	4899	4448
12/17/2017	AMB-1	No magnets	North	582	613	379	606	7906	12296
12/17/2017	AMB-1	10	South	614	644	439	1094	466912	489674
12/17/2017	AMB-1	10	North	645	675	454	625	256382	247786
12/17/2017	AMB-1	2	South	736	766	402	853	324366	319867
12/17/2017	AMB-1	2	North	676	704	461	227	828479	842695
12/18/2017	AMB-1	2	North	767	798	331	250	285239	284706
12/18/2017	AMB-1	2	South	799	829	684	670	448625	453199
12/18/2017	AMB-1	10	North	861	891	196	523	238264	246684
12/18/2017	AMB-1	10	South	830	860	401	736	713189	723051
12/18/2017	AMB-1	No magnets	North	892	921	98	151	562240	564348
12/18/2017	AMB-1	No Magnets	South	922	951	161	277	197194	190403
12/21/2017	AMB-1	No magnets	South	952	982	327	367	457677	462994
12/21/2017	AMB-1	No magnets	North	983	012	319	331	194719	191610
12/21/2017	AMB-1	2	South	013	042	968	677	520142	524373
12/21/2017	AMB-1	2	North	043	071	739	340	318219	307753
12/21/2017	AMB-1	10	South	073	103	334	255	360760	386158
12/21/2017	AMB-1	10	North	104	133	307	599	207896	192243
1/15/2018	<i>E. coli</i>	10	North	134	164	94	79	316369	320071
1/15/2018	<i>E. coli</i>	10	South	165	194	99	55	61240	57583
1/15/2018	<i>E. coli</i>	No magnets	North	225	255	132	68	390052	372658
1/15/2018	<i>E. coli</i>	No magnets	South	195	224	148	91	555242	555779
1/15/2018	<i>E. coli</i>	2	North	287	317	113	63	510621	509287
1/15/2018	<i>E. coli</i>	2	South	256	286	114	105	586177	594168
1/17/2018	<i>E. coli</i>	10	South	318	347	202	210	137087	157189
1/17/2018	<i>E. coli</i>	10	North	348	377	257	208	149807	144402
1/17/2018	<i>E. coli</i>	2	South	408	438	150	88	68338	64580
1/17/2018	<i>E. coli</i>	2	North	378	407	154	133	234014	236902
1/17/2018	<i>E. coli</i>	No magnets	South	470	500	173	177	345003	342536
1/17/2018	<i>E. coli</i>	No magnets	North	439	469	156	163	327040	339876
1/19/2018	<i>E. coli</i>	10	North	531	560	80	54	92394	82657
1/19/2018	<i>E. coli</i>	10	South	501	530	119	115	79074	82239
1/19/2018	<i>E. coli</i>	2	North	622	652	116	97	265464	255752
1/19/2018	<i>E. coli</i>	2	South	590	620	98	53	57900	61154
1/19/2018	<i>E. coli</i>	No magnets	North	684	715	79	30	201878	197894
1/19/2018	<i>E. coli</i>	No magnets	South	653	685	94	85	385572	390429

11 APPENDIX E: Autonomous counting of hanging drop data



The above is an example of the use of my autonomous counting program to count *E. coli* over the course of 20 minutes, sectioning the image to track changes in bacterial density over time. The clumping seen in the Section No. 5 was potentially caused by bacteria escaping the heat of the microscope bulb in the center of the meniscus, but no further study was performed to investigate the cause. Images taken of *E. coli* using the flat-bottomed well method showed no such edging behavior, although it should be noted that the observation period for flat-bottomed well experiments was five minutes rather than twenty minutes. Bacteria in images taken using the flat-bottomed well method did not have sufficient contrast from the background to perform autonomous counting; therefore, such sectioning analysis was not possible.